In the claims

Please see the attached sheets for the clean version of the claims.

Remarks

This is in response to an Official Action dated May 21, 2001. Reconsideration in view of the following is respectfully submitted.

A three-month extension of time is respectfully submitted herewith.

Claims 1-6 and 8-11 are pending. Claims 1-6 and 8-11 are canceled. Claims 12-14 are hereby added. New claim 12 is based upon original claims 1 and 6. New claim 12 is fully supported by the specification at the bottom of page 1 and at the top of page 2. All comments to the rejections are made in light of the new claims 12-14.

Claims 1-6 are rejected under 35 U.S.C. 112, first paragraph, for lack of enablement. In response, Applicants point out that the claims are given their broadest reasonable interpretation that is consistent with the specification. *See* §MPEP 2164.08.

As to the other *Wands* factors cited by the Examiner, the state of the prior art shows that p21 acts as a cell death preventing gene, wherein p21 prevents cell death by interfering with an endogenous apoptosis promoting gene. *See* A. McShea et al., Journal of Biological Chemistry, 275: 23181 (2000). The prior art shows that the expression of promoting gene by cellular stress or exogenous overexpression in the absence of p21 would result in cell death instead of survival.

Cell death is a general phenomenon in cell biology and plays a major role in a broad range of situations. Evidence from the literature shows that many genes induce apoptosis following their overexpression. The same holds true for therapeutic gene transfer by the use of viral vectors, wherein apoptosis can be the consequence of: a) the overexpressed gene itself and/or b) the vector employed for the therapeutic gene transfer. Thus, someone of ordinary skill in the art can assume from the state of the prior art that p21 when acting in combination with any selected gene, acts to prevent cell death, thereby resulting in prolonged cell survival, longer gene expression and a stabilization of gene expression in any type of gene that is selected.

12. (new) A method for improving the stable transfer of genetic material into mammalian cells , comprising the steps of

a) transferring a first vector carrying cDNA for p21 into a target cell

b) transferring a second vector carrying a selected gene, wherein a) precedes or occurs simultaneously with b), thereby resulting in a simultaneous expression of the p21 and the selected gene at a time when the second vector carrying the selected gene enters the cell.

13. (new) The method of claim 12, wherein the vector is chosen from the group consisting of retroviral, adenoviral, baculoviral, parvoviral vectors and herpes virus vectors.

14. (new) The method of claim 12, wherein the vector is adenoviral.

Further, the relative skill of those in the art would understand that p21 can be effective with any gene that is chosen, as evidenced by the prior art. As stated above, The prior art has shown that p21 acts an anti-apoptotic gene by interfering with an endogenous apoptosis promoting gene. *See* A. McShea et al., Journal of Biological Chemistry, 275: 23181 (2000). The promoting gene called CARB can be activated as a consequence of a disturbed cell cycle. The direct effect between this gene and p21 establishes p21's function to prevent any cell death induced by *endogenous* or *exogenous* stimuli.

In addition, gene transfer by either viral or non-viral vectors can induce cellular stress, as the prior art has shown that p21 can protect cells from the lethal consequences of inhibition of the cell cycle. *See* Bulavin et al., Deregulation of p21/p21Cip1/Waf1 pathway contributes to polyploidy and apoptosis of E1A+cHa-ras transformed cells after gamma-irradiation, Oncogene 18(41): 5611-9 (1999). The reduction of cell death induced by cellular stress and/or induction of cell cycle progression in the improper context would prevent the expression of a therapeutic transfer gene and kill the genetically modified cell, thereby rendering the transfer inefficient. P21 can prevent the induction of cell death, and

thus prolongs cell survival and maintains genetic stability of the p21-expressing cell.

Even though the Examiner cites numerous references on page 4, the references all cite to p21 acting alone, and not in combination with another gene. The instant application focuses on the expression of *both* a selected a gene *and* p21. The Examiner does not provide enough evidence that the combination is not enabled. The Examiner further points out that there is no direction or guidance to support the invention. However, the instant application provides an example using p21 and the gene for human alpha-1 antitrypsin in LoVo cells, wherein apoptosis of the cell was prevented in the presence of p21. See page 3 of specification. LoVo cells are cancer cells that originate from the human colon. In addition, there is no requirement for patent applications to show which mammalian cells are *not* appropriate targets. A requirement as such would be unreasonable and an undue burden. Furthermore, compliance with the enablement requirement does not turn on whether an example is disclosed. Applicants are not required to describe all actual embodiments. See §MPEP 2164.02. Thus, based on the aforementioned reasons, there is no undue experimentation and no lack of enablement.

Claims 1-6 and 8-11 stand rejected under 35 U.S.C. 112, second paragraph, for being indefinite. The claims are canceled, and new claims 12-14 are added. Claim 12 recites steps a) and b), wherein a) may precede or occur simultaneously with b), to result in a simultaneous expression of both p21 and the selected gene. New claim 12 is fully supported by the specification starting at page 1, lines 22-26 to page 2, lines 1-2. The use of p21 with a selected gene improves the transfer of genetic material, because the previous methods were limited by the induction of apoptosis, or programmed cell death. p21 appears to have a role in preventing apoptosis, thereby allowing greater efficiency in the transfer of genetic material. See page 2 of specification.

Claims 1-3, 5, and 9-11 stand rejected under 35 U.S.C. 102(b) as being anticipated by Nabel. Nabel teaches the inhibition of malignant cells by p21. The reference does not teach the simultaneous co-expression of p21 and the selected gene with immunotherapy, gene therapy, proteins, prodrug converting enzymes or anticancer drugs. The reference only mentions selection markers such as antibiotic resistance genes, wherein the genes are used for the *sole purpose* of selecting successful transfectants. See pg. 6, lines 24-6. Furthermore, the reference relates to the treatment of tumor suppression and

restenosis. See page 3, fourth full paragraph. Nabel describes the use of p21 in combination with immunotherapeutic agents, gene therapy, proteins, prodrug converting enzymes and anticancer drugs, but they are used for the treatment of restenosis, not the purpose cited in the instant application. Since anticipation requires a single reference to teach each and every element, the claims are not anticipated.

Claims 1,2,4 and 5 are rejected under 35 U.S.C. 102(b) as being anticipated by Kokunai. Kokunai discloses a differentiation of a human glioma cell line having mutant p53 as being induced by transfection of p53 and p21 cDNAs. Expression of the neomycin-resistant gene is used solely for the purpose of obtaining stable transfected cell lines. P21 is not co-expressed for the stabilization or improvement of the expression of the gene. Rather, its expression is selected for and maintained by the addition of the antibiotic G418 to the culture medium. This is evidenced by the method for the generation of the T-98G/p53 cell line which was transfected with an expression vector (pCDM8-p53/neo) that simultaneously expresses *p53*, instead of p21, and the neomycin-resistant gene. See page 643. P53 had been transfected into the cells before the transfection of p21. Thus, p21 could not have been used to stabilize or improve the transfer of p53 into the glioma cell line.

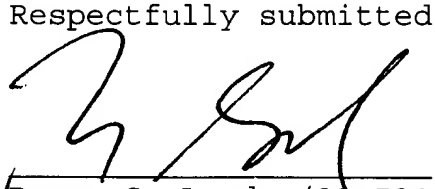
The Examiner rejects claims 1,2 and 5 under 35 U.S.C. 102(b) as being anticipated by Mudryj. Mudryj teaches a method for detecting a senescent state in a cell by detecting a p21-E2F complex. The reference does not address the same purpose as the instant application. Its purpose is to detect cells in a specific state. The reference does not employ the co-transfer of two expression vectors that lead to the simultaneous or sequential expression of two different transfer genes. Furthermore, the reference does not assay the effect of p21 expression on the stability and expression of the second co-expressed gene. In contrast, the instant application seeks to improve the transfer of genetic material using p21 and a selected gene to result in a simultaneous expression of both the gene and p21. Thus, the claims are not anticipated.

Claims 1-5 and 8-11 stand rejected under 35 U.S.C. 103(a) in view of Nabel. Nabel was described above. The reference addresses a purpose different from the instant application. The method in Nabel seeks to treat cancer and restenosis. In contrast, the instant application provides an improved method to generally facilitate gene transfer in order to prevent the induction of apoptosis and thus prolong the expression of the selected gene. See page 1, lines 18-21 of specification.

Someone having ordinary skill in the art would not look to a reference having a different purpose, because the reference does not address the same concerns. Thus, the claims are not rendered obvious.

Wherefore, allowance of all pending claims is earnestly solicited.

Respectfully submitted,



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Marked up version of Specification

At page 4, please delete the first paragraph and insert the following after the heading for Figure 1:

-- Replication-deficient recombinant adenovirus vector induces apoptosis by uncoupling of S-phase and mitosis. The corresponding flow cytometry analysis of cell cycle distribution (Figure 1A[,]-1B) and in situ detection of apoptosis by TUNEL-assay (Figure 1C[,]-1D) are demonstrated for LoVo cells 48 h after Ad vector infection. Cells were mock infected (buffer control) (Figure 1A, Figure 1C) or infected with an Ad vector carrying alpha-1 antitrypsin (100 plaque forming units per cell) (Figure 1B, Figure 1D). --

At page 4, please delete the second paragraph and insert the following the heading for Figure 2:

-- Overexpression of p21 prevents adenovirus-induced apoptosis. In situ detection of apoptosis in LoVo cells 48 h after infection. Cells were mock infected with buffer (Figure 2A, Figure 2D), infected with an Ad vector carrying the human alpha-1 antitrypsin at a dose of 100 plaque forming units per cell (Figure 2B, Figure 2E) or infected with an Ad vector coding for p21 (100 plaque forming units per cell) (Figure 2C, Figure

2F). Shown are representative photographs at a magnification of 200-fold (Figure 2A-2C[A-C]) and 600-fold (Figure 2D-2F[D-F]).--

At page 4, please delete the third paragraph and insert the following the heading for Figure 3:

-- P21 protects against adenovirus-mediated apoptosis by prevention of a G2-like arrest. Demonstrated is the flow cytometry analysis of cell cycle distribution of LoVo cells after 48 h of infection with different doses of Ad vectors expressing either the cDNA of human alpha-1 antitrypsin (_) or the cDNA of p21 (_). Shown are the relative percentages of the cell populations which are in the G_0/G_1 or G_2/M phase of the cell cycle as well as the percentage of living cells in the whole population (negative in the propidiumiodid staining; PI^-). The data represent the mean ± standard error of three experiments.--